EPIGENETIC REGULATION BY EARLY ADVERSE EXPERIENCES IN THE NICU

BIOCHEMICAL PATHWAYS TO PRETERM INFANTS’ OUTCOMES

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Infants with neurodevelopmental disabilities (cerebral palsy, genetic syndromes, severe prematurity, psychomotor delay, pediatric tumors)
Presentation
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Behavioral Epigenetics
Mother-infant interaction

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Technological applications
Presentation
0-3 Center - Mission

(RE-)HABILITATION

RESEARCH
INNOVATION
CARE
A. Preterm birth and NICU stress
B. Behavioral epigenetics principles
C. Preterm Behavioral Epigenetics Project
D. Take-home messages
Very preterm (VPT) birth (gestational age < 32 weeks) is a major risk factor for human infants development (Blencowe et al., 2012). Even in absence of severe neurobehavioral injuries or morbidities, VPT infants are long-lasting hospitalized in the NICU (Lester, 2011).

NICUs are highly medical and technological environments where VPT infants are exposed to maternal separation and different sources of physical (lights and sounds), invasive (ventilation) and painful (skin-breaking procedures) stimulations (Greisen et al., 2009; Grunau et al., 2006; Grunau, 2013; Brummelte et al., 2012).

Developmental Care (DC) interventions (Als, 2011; Flacking et al, 2012; Welch et al., 2014) are family-centered strategies meant to promote parental involvement, early physical contact, emotional bonding, breast-feeding, infants’ neurobehavioral stability and parenthood transition.
Pain-related stress exposure in NICU
Effects on brain and neuroendocrin

Ranger et al (2013)
Provenzi et al (2016)
Developmental Care effects
Data from multi-centric study (k = 25)

- Montirosso et al (2011)
  - NNNS (discharge)

- Montirosso et al (2013)
  - Depression (discharge)

- Montirosso et al (2016)
  - Quality of Life (5 years)

The Neonatal Adequate Care for Quality of Life (NEO-ACQUA) study
Assessment tool: Quality of Care Checklist [quantitative]

Behavioral problems (18 months)

- T0 (Measurement of DC)
- T1 (NICU discharge)
- T2 (6 months)
- T3 (18 months)

Montirosso et al (2017)
The missing link
How do NICU exposures leave a trace…?

<table>
<thead>
<tr>
<th>NICU-related stress</th>
<th>NICU-related care</th>
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<tbody>
<tr>
<td>HPA axis stress dysregulation (Provenzi et al., 2016).</td>
<td>Better HPA axis stress regulation (Kleberg et al., 2008).</td>
</tr>
<tr>
<td>Poor neurobehavior at discharge (Lester et al., 2011).</td>
<td>Better neurobehavioral profile at discharge (Montirosso et al., 2012).</td>
</tr>
<tr>
<td>Higher risk of behavioral problems (Chau et al., 2014).</td>
<td>Reduced risk of behavioral problems (Montirosso et al., 2018).</td>
</tr>
<tr>
<td>Altered brain growth (Ranger et al., 2015; Smith et al., 2011).</td>
<td>Better brain growth (Als et al., 2004).</td>
</tr>
</tbody>
</table>

But which are the mechanisms linking early NICU-related exposures (for bad and for good) with developmental trajectories and phenotypic outcomes?
What makes a good rat mama
How the care environment gets under the skin
More than your genes
Why your DNA is not your destiny

Genetic Mutations Often Alter Meaning
A gene’s nucleotides (code letters) form a blueprint for a protein (top). A wrong letter or other mutation can alter the resulting protein (bottom) or cause too much or too little to be made.

Epigenetic Changes Alter Activity
Chemical tags known as epigenetic marks sit atop genes, either on the DNA itself or on the histone proteins around which DNA is wrapped (below). Changes in the mix of these marks can alter a gene’s behavior, turning the gene off, so that protein synthesis is inhibited, or turning it on—all without changing the information the gene contains.
DNA methylation

Switching DNA on and off
Methylation changes in specific genes
Candidate loci of environmental susceptibility?

**NR3C1**
Gene encoding for the glucocorticoid (cortisol in human) receptors in the brain. Cortisol is involved in developmental regulation of:
- Cognitive functions
- Memory formation and consolidation
- Emotional behavior
- Stress response and reactivity

**SLC6A4**
Gene encoding for the serotonin transporter. Serotonin transporter are involved in developmental regulation of:
- Cognitive functioning
- Memory consolidation
- Emotional behavior
- Stress regulation
Figure 2. A prospective model to inform preterm behavioral epigenetic studies.
SLC6A4 methylation

Biomarker of adversities in humans

Devlin et al., 2010  Duman & Canli, 2011  Essex et al., 2013

Provenzi et al (2017)
**The PBE project**
**Study design and aims**

\[ n = 64 \]  VPT infants (excluding major neonatal and perinatal complications)

\[ n = 56 \]  Full-term (FT; gestational age > 37 weeks) healthy infants
Results [1]

Effects of early pain-related stress on SLC6A4 methylation in VPT infants

VPT infants split in low (LPE) and high (HPE) pain exposure based on median value of skin-breaking procedures count.

Aim #1: no differences in SLC6A4 CpG-specific methylation at birth among VPT-LPE, VPT-HPE and FT infants.

Aim #2: Significant birth-to-discharge increase in SLC6A4 methylation only in VPT-HPE infants.
Results [2]
 Effects of SLC6A4 methylation increase on temperament at 3 months

Aim #3: increased CpG-specific SLC6A4 methylation at discharge linked with less orienting and approach in VPT infants. No significant associations in FT infants.
Results [3]

Long-term effects: Developmental Quotient at 12 months via brain alterations

From early stress to 12-month development in very preterm infants: Preliminary findings on epigenetic mechanisms and brain growth

Monica Fumagalli, Livio Provenza, Pietro De Carlo, Francesca Desimone, Ida Siriguanni, Roberto Giordano, Claudia Cimovsky, Letizia Squarcina, Uberto Pozzoli, Fabio Truzzi, Paolo Brambilla, Renato Borgatti, Fabio Mora, Rosario Montirossi
More than methylation
The anti-parallel DNA and telomere issue

DNA is antiparallel

The DNA and RNA can only be attached from 5' to 3', starting onto the 3' position

Helicase, DNA helix breaker

Single-strand binding proteins
More than methylation
The anti-parallel DNA and telomere issue

The DNA is antiparallel.
The DNA and RNA can only be attached from 5’ to 3’, starting onto the 3’ position.

The DNA Okazaki fragments are primed by an RNA primer, which is finally replaced with DNA by a replacement enzyme. The fragments are binded by a ligase enzyme. Nonetheless, the final gap will be not binded and a specific series of nucleotides will not copie and it will be lost.
Telomere length (TL) erosion
Biomarker of cellular aging and stress

Key elements of telomere regulation
1. At each cell division, telomere length (TL) shortens;
2. The Hayflick limit defines the total available number of cell divisions, aka the individual minimum critical TL;
3. Indeed, the rate of telomere erosion is considered to be a biomarker of cellular aging.
PBE project + telomeres
Recap of study design and TL-related aims

VPT birth
VPT discharge
Follow-up session

< 32 GA weeks
> 37 GA weeks
3-month-old (CA for VPT infants)

VPT birth
VPT discharge
Follow-up session

< 32 GA weeks
> 37 GA weeks
3-month-old (CA for VPT infants)

Aim 1: birth-related TL difference between VPT and FT infants?

Aim 2: pain-related effect on TL in VPT infants?

Aim 3: TL effect on VPT and FT infants salivary cortisol stress regulation?

n = 46   VPT infants (excluding major neonatal and perinatal complications)
n = 31   FT healthy infants
From NICU to HPA axis programming


Provenzi et al (2019)
Behavioral Epigenetics

Why should we be interested in this stuff?

What Behavioral Epigenetics can give to us

1. Better understanding of early life exposures
2. Unveiling biological underpinnings of developmental programming
3. Contributing to evidence-based support for early interventions
4. Supporting complex view of human development
5. Advancing the nature vs. nurture field of studies

What we can give to Behavioral Epigenetics

1. Guiding the wide-spread of research in the field
2. Providing relevant questions for clinical issues
3. Providing interpretations of findings in context
4. Investing properly research funds into relevant directions
5. Integrating multiple epigenetics biomarkers in a developmentally oriented way
NICU-related epigenetic regulation
Emerging linking mechanisms

1. From NICU to DNA

NICU-related stress associates with environmental-driven regulation of methylation status of specific gene associated with behavioral and socio-emotional development (SLC6A4) and TL shortening.

2. From DNA to outcome

Increased SLC6A4 methylation and TL shortening associate with differential less-than-optimal outcomes in VPT infants, including behavioral difficulties, socio-emotional stress hyper-reactivity, and HPA axis dysregulation.

3. What’s next?

We are looking forward to
A. Long-term trajectories up to 4 years of age;
B. Epigenetic correlates of DC practices;
C. Epigenetics of neural mirroring system in VPTs;
D. Key role of NICU touch in VPT infants’ epigenetic regulation.
Behavioral epigenetics in VPT infants

Study design advantages

Retrospective study design

- Subjective report bias
- Correlational, not predictive
- Limited moderation analysis
- Limited control of confounders

Prospective study design

- Less subjective report bias
- Causal links possible
- Supports moderation analysis
- Supports control of confounders
What about resilience? Epigenetics and protective factors

- Maternal touch
- Maternal breastfeeding
Ongoing now
Epigenetic vestiges of an early parental intervention using video-feedback
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